Indian Council of Medical Research



TECHNICAL REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1955

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CONTENTS

LEPROSY.

PLAGUE.

CLINICAL RESEARCH.

MATERNITY AND CHILD WELFARE.

FILARIASIS.

VIRUS DISEASES.

I. COMPOSITION OF THE SCIENTIFIC ADVISORY BOARD.

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III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1955.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Baroda on the 21st and 22nd December, 1954 and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 28th March, 1955. *The views expressed by the individual workers are not necessarily the views of the Council.*

CHOLERA

1. Inquiry on the mutation of cholera vibrios in fish under Dr. M. N. Lahiri at the All-India Institute of Hygiene & Public Health, Calcutta.

The objects of the inquiry were to study the possibility of mutation of cholera vibrios in the guts of fishes and to determine the serology of NAG suspected to be mutants, as also the phage typing of the original *V. choleræ* cultures and NAG.

Artificial infection experiments.—During the year under report ten series of artificial infection experiments were conducted with four species of hardy fishes, viz., the climbing perch (*Anabus testudineus*), the cat fishes (*Heteropneustes fossilis*) and (*Clarias magur*) and the murrel (*Ophicephalus punctatus*). Specimens obtained from their natural habitats were kept under observation in the laboratory till they ceased to excrete vibrios. It was found that these species of fish continued to excrete vibrios for a period of 4 to 5 months. This corroborates the observations reported last year regarding the natural excretion of NAG vibrios by the fish.

In artificial infection experiments, specimens rendered vibrio-free for a period of about 5 months were infected with both Ogawa and Inaba sub-types of *V. choleræ*. All the specimens excreted agglutinating vibrios for a period of three to four days subsequent to the infection. In each case, they excreted NAG vibrios for varying periods of time, within a maximum of 15 days after infection. The NAG excreted in the initial stages invariably belonged to Heiberg's group VI. In a few cases NAG of groups I and II were excreted after a few days but it is interesting to note that in every case the NAG excreted vibrios of this group only.

With a view to ascertain whether artificial infection with cholera vibrios would stimulate the excretion of NAG vibrios which may be retained in the guts of fishes presumed to be vibrio-free in the experiments, artificial infection was tried with other pathogenic oragnisms, viz., *Salmonella typhi* and *Sh. flexneri*. But none of the infected fish excreted any NAG vibrios. These experiments lend further support to the surmise that *Vibrio choleræ* may actually be undergoing some change in the guts of fishes.

Serological studies of NAG vibrios of group VI isolated after artificial infection were conducted. It was observed that the group VI vibrios obtained from the same species of fishes were serologically identical. But the same group isolated from different species showed serological differences. Similar studies of groups I and II vibrios are now in progress. Further work will be required to understand the significance of this

Modification of the technique of isolation.—It was reported last year that comparative studies of culture techniques have indicated that the cultures should be incubated for 48 to 72 hours to obtain satisfactory results. Further studies were made during the year under report using cholera stools from cases admitted in the N. R. Sarkar Medical College Hospital as also water and gut content samples from fish. Cultures which were negative after 24 hours' incubation showed good growth of vibrios after 48 and 72 hours' incubation. A pH of 8·4 was found to be most suitable for the growth and survival of vibrios. In view of these findings it will now be necessary to re-examine the data collected during previous years regarding the absence of cholera vibrios in different sources.

Phage studies.—The phage typing of the vibrios is under progress and when a sufficient number of pure line phages have been obtained these will be utilized to study whether there is any relationship between the parent agglutination strains and the NAG strains obtained after artificial infection.

2. Inquiry into the state of anuria and uræmia in cholera with special reference to its treatment under Dr. A. Das at the Nilratan Sircar Medical College, Calcutta.

A series of 20 normal controls (healthy medical students) were investigated with a view to determine the normal values of sodium, potassium and chloride in plasma. The results are as follows:—

		In meqL Highest	In meqL Lowest	In meqL Average
Na	...	140·8	137	139·1
K	...	5·1	4·6	4·9
Cl	...	94·6	88·7	90·1

The cases of uræmia selected for this investigation were from the following groups:—

(A) Cases of cholera admitted with a degree of dehydration and collapse (radial pulse imperceptible) which suggested the possibility of development of uræmia. They had no clinical manifestations of uræmia at the time of admission—60 cases.

(*i*) Those that did not develop uræmia subsequently—42 cases.

(*ii*) Those that developed uræmia—18 cases (10 deaths).

(B) Similar cases but admitted with persistent anuria for at least 25 hours—5 cases (1 death).

(*i*) Admitted with clinical manifestations of uræmia—1 case.

(*ii*) Developed uræmia subsequently—4 cases.

(C) Those who developed obvious clinical manifestations of uræmia during treatment—22 cases (18 cases from group A and 4 cases from group B).

Thus, the total number of uræmia cases investigated was 23 including the case admitted with clinical manifestations of uræmia.

All the cases had the usual clinical manifestations of uræmia in varying degrees. One feature of interest was the presence of extra-cellular over-hydration developing during the course of treatment in 14 cases of this series. This was clinically manifested in six cases by engorgement of the veins of the neck (rise of venous pressure was confirmed by manometric measurements), puffiness on the eye-lids, sacral œdema and rise of body-weight. Over-hydration in each of these six cases was confirmed by fall of plasma specific gravity and hæmatocrit values. In eight other cases the presence of extra-cellular over-hydration was evident from the reduction of the plasma specific gravity and hæmatocrit values although the clinical manifestations of over-hydration had not yet appeared.

Biochemical findings in cases which were clinically diagnosed to be in a state of uræmia:

Sodium and potassium content of plasma.—Taking normal sodium of plasma to be between 130 and 143 meqL and normal potassium content to be between 4·1 and 5·6 meqL (Hawk *et al.*, 1947, ' Practical Physiological Chemistry ').

		Cases.
1.	Normal sodium with	
	(*i*) Normal potassium in plasma ...	7
	(*ii*) Low potassium in plasma	1
2.	High sodium with	
	(*i*) Normal potassium in plasma	4
	(*ii*) High potassium in plasma	1
3	Low sodium with	
	(*i*) Normal potassium in plasma .	8
	(*ii*) High potassium in plasma .	2
Blood urea		
	(*i*) Below 70 mg. per cent	4
	(*ii*) Between 71 and 120 mg. per cent ...	14
	(*iii*) Above 120 mg. per cent ...	5

Plasma bicarbonate content

Between 22·4 c.c. and 43·68 c.c. of CO_2/100 c.c. of plasma in all cases (normal bicarbonate content of plasma, 53 c.c. to 75 c.c. of CO_2/100 c.c. of plasma).

Blood inorganic phosphate content

Between 4·5 and 17·6 mg. per cent (normal inorganic phosphate content of blood 3·7 mg. per cent).

[illegible] the plasma of 20 [illegible] cases of uræmia in [illegible] ring the course of [illegible] The study showed [illegible] of [illegible] ations [illegible] in [illegible] the

23 cases died.

3. Inquiry on the evaluation of the phages acting on vibrios and the application of bacteriophage typing in epidemiological investigations on cholera under Dr. B. Ghosh Ray and Dr. M. N. Lahiri at the All-India Institute of Hygiene & Public Health, Calcutta.

Object.—To isolate different phages from cholera stool and study their lytic effect on *V. choleræ* and non-agglutinable (NAG) vibrios. This is essential to evolve a phage typing method which may be helpful in epidemiological investigations of cholera. The use of phages will also be made to elucidate the interrelationship between the *V. choleræ* and other NAG vibrios usually excreted by cholera cases.

Synopsis of work done.—Preliminary work was done on the standardization of media for isolating *V. choleræ* including the phage-carrying strains and NAG vibrios from cholera cases.

In order to find a simple way to handle large number of fæcal specimens either in hospital or in field practice, rectal swabs obtained either directly from patients or dipped in catheter specimens of the liquid stool, were employed for inoculating media. It was observed that even during the acute stage of the disease, simultaneous use of the bile-salt agar plates (pH 8·4) and the Wilson and Blair's liquid medium increased the chance of positive isolations. Two hundred and sixty samples of stool were examined. By direct plating method positive isolations were obtained from only 45 per cent of the samples. On the other hand, the combined method gave 58·8 per cent positive isolations. The effects of pH in Wilson and Blair's liquid medium, the period of incubation and the volumes of the media and of the inoculum are being studied further. The technique will be tried during the next recrudescence of the disease.

Swabs dipped in catheter specimen yielded positive isolations in 61 per cent cases, whereas the rectal swabs obtained directly were positive in 55 per cent cases. With further improvement in the technique, it is possible that employment of rectal swabs alone will be of great help. The strains of *V. choleræ* isolated during the last epidemic have been found to be of Inaba sub-type only. These and other NAG vibrios are being maintained for future investigations.

Fifteen phage strains have been obtained from cholera stools. Strains of *V. choleræ* including those carrying phages are being studied for further isolations. Experiments are in progress to obtain 'pure' line phages and determine their nature. So far, all the phages from stool filtrates are being propagated in association with the corresponding strain of *V. choleræ* isolated from the same samples of stool.

A start has been made to find out a convenient method to lyophilise the large number of vibrio strains already isolated as well as the phage preparations.

MALARIA

I. Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

A. Laboratory investigations.

During the period under review, investigations on the following problems were carried out:—

1. Solubility of DDT crystals in the cuticular wax of houseflies and mosquitoes (Continued).

2. Investigations on the development of resistance in house-flies and mosquitoes to chlorinated hydrocarbon insecticides (continued).

3. Development of sporogony cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT (continued).

4. Prevention of sorption of insecticides into mud surfaces (continued).

5. Efficacy of newer insecticides such as Malathion and Diazinon.

B. Field investigations.

Comparative effectiveness of DDT, BHC, Dieldrin and Malathion, residual sprays against mosquitoes.

C. Samples of insecticides and spraying equipment tested.

D. Publications.

E. Proposed plan of work 1956–57.

A. Laboratory investigations.

1. *Solubility of DDT crystals in the cuticular wax of house-flies and mosquitoes. Physical properties of cuticular lipoids.*

It was observed that the dissolution of DDT crystals in the cuticular wax of *M. nebulo* and *A. stephensi* started almost immediately and the majority of the crystals disappeared in three and six hours, respectively. In the case of *C. fatigans* crystals are observed intact even after 24 hours.

the body (without wings) and from the legs were tested for their physical properties. The colour of the waxes from the legs was dark and that from the body dark brown. The various physical properties were as follows:—

Physical properties	*Musca*		*Culex*	
	Body	Legs	Body	Legs
Slipping point	42°C.	55°C.	51°C.	60°C.
Melting point	45°C.	60°C.	52°C.	65°C.
Solidification	40°C.	53°C.	50°C.	58°C.
Refractive index ...	1·3852	1·4121	1·4512	1·4623

It was evident from data obtained that the waxes from the legs of both flies and mosquitoes melted at higher temperatures than the waxes from the body and those extracted from *Culex* had a higher melting point than the one extracted from *flies*. It is possible that this physical property is responsible for the natural biological resistance of *Culex* to DDT.

2. *Investigations on the development of resistance in house-flies and mosquitoes to chlorinated hydrocarbon insecticides.*

Reports from some parts of the world allege that in certain areas where DDT has been in use for a number of years, some species of mosquitoes have developed resistance to DDT and other allied insecticides. This has drawn the attention of all public health workers to the possibility of such a phenomenon interfering with the nation-wide programmes for the control of malaria by DDT used as a residual insecticide.

From the reports of successful malaria control obtained in various parts of India by DDT residual spray, it is concluded that so far anophelines vectors of malaria in India have not developed any appreciable degree of resistance although DDT has been in use in certain areas for over eight years. In order to study whether or not even a small amount of resistance has so far developed in some of the species, specific investigations were carried out in Northern India (States of Delhi and U.P.).

A. culicifacies collected from an area sprayed with DDT for over seven years was subjected to tests in accordance with the procedure laid down by the World Health Organization (Technical Report Series No. 80). These mosquitoes were found to be as susceptible to DDT as those from an unsprayed area. From the complete absence of *A. fluviatilis* and the absence of malaria transmission in sprayed areas in which investigations were carried out it is safe to conclude that no resistance has so far developed in U.P. Terai. Similar investigations are being carried out on other vector species in other parts of the country. In order to get as much information as possible within a very short time details of this technique and a testing kit has been forwarded to various workers for detecting if any resistance has developed in areas where DDT or other chlorinated hydrocarbon insecticides have been in use for a number of years.

Unlike anophelines, *Culex fatigans* have been found to have developed a certain degree of resistance in some parts (Delhi, U.P., and Madras). Studies on this problem are being continued.

The susceptibility of normal *A. culicifacies*, *A. subpictus*, *Culex fatigans* and *house-flies* to DDT has been determined so as to get a base line data which may be useful for purposes of comparison with similar data obtained from areas sprayed with DDT. The results are tabulated as under:—

Per cent mortality after one hour exposure*.

Species	Concentration of DDT 0·25 per cent	0·5 per cent	1·0 per cent	2·0 per cent
A. culicifacies	45·0	65·9	97·4	100
A. subpictus	46·6	61·1	94·8	100
House-flies	73·7	75·7	89·4	95·4
Per cent mortality after 24 hours exposure.				
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Similar studies have been carried out on the 4th instar larvæ of *Ædes ægypti* and *Culex fatigans.* The larvæ were exposed to DDT concentrations of 0·01 to 5·0 parts per million and BHC gamma isomer concentrations of 0·1 to 1 part per million. The results with *Ædes ægypti* are fairly consistent and the M.L.D. 50 for DDT and BHC appear to be about 0·02 ppm. The results with *Culex fatigans* are variable and need further confirmation.

Besides the above, it is also being investigated as to whether (1) the houseflies and mosquitoes exposed to DDT and BHC separately and to DDT and in BHC combination would develop resistance to DDT and BHC simultaneously; and (2) whether the development of resistance will be hastened if the same insecticide is used both as a larvicide and as an adulticide?

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Development of sporogony cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT.

Very little is known as to what happens to oocysts or to sporozoites e body of resistant and normal strains of mosquitoes after exposure T. Studies were undertaken at Coonoor on this problem and a few

It was evident from data obtained that the waxes from the legs both flies and mosquitoes melted at higher temperatures than the wax from the body and those extracted from *Culex* had a higher melting poi than the one extracted from *flies*. It is possible that this physical proper is responsible for the natural biological resistance of *Culex* to DDT.

2. *Investigations on the development of resistance in house-flies and mosquitoes chlorinated hydrocarbon insecticides.*

Reports from some parts of the world allege that in certain are where DDT has been in use for a number of years, some species of mosquito have developed resistance to DDT and other allied insecticides. This h drawn the attention of all public health workers to the possibility of suc a phenomenon interfering with the nation-wide programmes for th control of malaria by DDT used as a residual insecticide.

From the reports of successful malaria control obtained in variou parts of India by DDT residual spray, it is concluded that so far anophelin vectors of malaria in India have not developed any appreciable degree resistance although DDT has been in use in certain areas for over eig years. In order to study whether or not even a small amount of resistanc has so far developed in some of the species, specific investigations we carried out in Northern India (States of Delhi and U.P.).

A. culicifacies collected from an area sprayed with DDT for ove seven years was subjected to tests in accordance with the procedure lai down by the World Health Organization (Technical Report Series No. 80) These mosquitoes were found to be as susceptible to DDT as those from a unsprayed area. From the complete absence of *A. fluviatilis* and the absenc of malaria transmission in sprayed areas in which investigations wer carried out it is safe to conclude that no resistance has so far develope in U.P. Terai. Similar investigations are being carried out on other vect species in other parts of the country. In order to get as much informatio as possible within a very short time details of this technique and a testin kit has been forwarded to various workers for detecting if any resistanc has developed in areas where DDT or other chlorinated hydrocarbo insecticides have been in use for a number of years.

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Per cent mortality after one hour exposure*.

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3. *Development of sporogony cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT.*

Very little is known as to what happens to oocysts or to sporozoites in the body of resistant and normal strains of mosquitoes after exposure to DDT. Studies were undertaken at Coonoor on this problem and a few

limited observations on the viability of gametocytes in fowls poisoned by oral administration of DDT were also recorded.

A DDT-resistant and a normal strain of *C. fatigans* for *P. relictum* in local sparrows, a DDT-resistant and a normal strain of *A. fluviatilis* for human malaria and only a normal strain of *Aëdes* (*Stegomyia*) *aegypti* for *P. gallinaceum* in domestic fowls were used.

From the data available, it is evident that mere development of insecticidal resistance which was high in *C. fatigans* and low in *A. fluviatilis*, did not result in any concomitant change of the vectorial capacity of these two species.

In the case of *Aëdes aegypti* also—it was seen that a sublethal exposure to DDT preceding or succeeding infective blood meal had no effect on the development of sporogony cycle of *P. gallinaceum* or on the sporozoites. Sporozoites in infective *Aëdes aegypti* were not rendered non-infective by a sublethal contact with DDT, nor were impaired antigenically when sporozoite agglutination was studied.

Experiments with fowl indicate that in a normal fowl poisoned by DDT, the parasites multiplied and produced viable gametocytes which on being ingested by mosquitoes resulted in the production of sporozoites which, in turn, were proved to be infective to normal fowls.

4. *Prevention of sorption of insecticides into mud surfaces.*

(*a*) Correlation between the amount of insecticides sorbed into mud surfaces, to the porosity of the surfaces was studied. Panels with varying porosity ranging from 9·2 to 19·2 per cent were prepared by mixing different quantities of sand with mud and were dried in shade for a week under identical conditions. Dried panels were sprayed with 75 per cent DDT water-dispersible powder at the rate of 100 mg. DDT per sq. ft. The panels were allowed to dry for 24 hours after which scrapings from 25 sq. cm. surfaces were removed and the amount of DDT present in them determined by using a spectrophotometer. The results are briefly summarized below:—

Porosity	Residual DDT in scraping	DDT mg./sq. ft.
9·20	2·44	87·84
10·65	2·28	81·88
12·38	2·22	79·92
14·15	1·89	68·04
17·43	1·80	64·80
18·90	1·78	64·08
19·20	1·76	63·37

From the above table it would appear that absorption of DDT into mud increased with the increase in porosity. Further work is in progress on panels with the same chemical composition but of different porosity.

(*b*) *Studies on mud blocks received from Afghanistan.*—The World Health Organization sent a number of mud blocks from Afghanistan for detailed

study regarding the sorption of insecticide in these blocks and the physical and chemical properties of the mud are as follows:—

Physical properties		Chemical properties	
	Per cent		Per cent
1. Specific gravity ...	2·56	Calcium carbonate ..	7·25
2. Porosity	9 6	Bicarbonate ..	0·0427
3. Permeability ...		Total quantity of iron, phosphorus and aluminium oxides ..	13 22
4. Sedimentation* ...		Iron oxide (Fe_2O_3) ..	7 79
Time, minutes	Per cent sedimentation	Phosphorus pentoxide (P_2O_5) ..	0·60
10	17	Aluminium oxide (Al_2O_3) ..	4·83
20	30	Insoluble silica (SiO_2) .	38·87
30	52		
60	66		
120	74		
210	80		
300	83		

*The sedimentation was determined of the ground mud which was passed through a 16 mesh (ASTM) sieve. Thirteen per cent coarse material remained on 16 mesh sieve

The mud blocks were sprayed with DDT suspension at the rate of 100 mg./sq. ft. and scrapings from 25 cm. area were removed 24 hours after spraying and once every week from the same block. The biological effectiveness of the deposits was also determined simultaneously The quantity of DDT estimated from the scrapings and percentage mortality figures are given below:—

Age of deposit	DDT/mg. sq ft.	Biological effectiveness against *C. fatigans*: 30 minutes Exposure		60 minutes Exposure	
		per cent mortality after 24 hours	after 48 hours	per cent mortality after 24 hours	after 48 hours
24 hours	64·4				
week					
1	53·28	..	...	...	...
2	30 5	...	...	...	...
3	4·5	82·5	100	92·5	100
4	28·44	79·5	93·7	96·5	100
5	4·5	65 0	86 7	93·7	95·0
6	16·92	48·7	67·5	77·5	85·0
7	15·12	25·0	35·0	38·75	62·0
8	10 8	12·5	22·5	25·0	40·0
9	10·6	7·5	17·5	...	...
10	8·58	2·5	12·5	...	...
11	0·294	0	0	...	...

There appears to be a close correlation between the amount of DDT present on the surface and the biological effectiveness of the deposit. Due to the presence of high iron-oxides content deposit of DDT 200 mg/sq. ft. was effective only for about eight weeks.

Micro-photographs from the same spot of the block were also taken every week. These definitely show the gradual disappearance of crystals after two weeks of application, but subsequently dust particles interfered with the results.

(*c*) *Measures to prevent sorption of insecticides into mud surfaces.*—As reported last year, glue and potassium dichromate mixture, glue and soap mixture and Ripolin clear coatings were tried to prevent sorption of insecticides into mud surfaces but none of these materials had been found satisfactory. In the light of further discussion with Technical Development Establishment Laboratory (who made the suggestion for the use of glue and potassium dichromate mixture) tests were repeated with slight modifications.

The modified formulæ used were as follows:—

1. Glue pot dichromate solution:
   Glue 6·0 parts.
   Potassium dichromate: 0·5 parts.
   Water: 93·5 parts.
2. Glue and soap solution:

Glue	...	...	1 lb.
Sunlight soap	...	...	½ cake.
Water	...	...	1 gallon.

Uniform mud blocks (6″×6″×½″) were treated with dichromate glue solution at the rate of 12·5 (750 mg.) and 25 c.c. (1,500 mg.) per sq. ft., and glue soap solution at the rate of 2 c.c. (250 mg.) and 4 c.c. (500 mg.) per sq. ft. The panels were dried for 48 hours and one set was then sprayed with DDT suspension at the rate of 50 mg./sq. ft., and another set with DDT emulsion supplied by the T.D.E.L. at the same rate. The test insects, *Culex fatigans* and *Musca nebulo*, were exposed to the treated surfaces for 15 minutes and were then kept for 24 hours under observation in clean cages. A new set of panels was used after every four weeks. These experiments are being continued as it was pointed out that mosquitoes at certain times of the year are more susceptible than at others. The data so far collected showed a slight increase in the duration of residual effectiveness but only by about a week or so.

5. *Efficacy of newer insecticides such as Malathion and Diazinon, etc.*

During the period under review the following newer insecticides of the phosphate series were tested for their biological effectiveness against houseflies and mosquitoes:

(*a*) *Malathion.*—$C_{10}H_{19}O_6$ PS_2, is the name given to the insecticidal chemical O—O—dimethyl— (1, 2— dicarboxy ethyl) dithiophosphate, belonging to phosphate series of compounds. It has been reported that unlike other compounds of the phosphate series it can be safely handled and is less hazardous.

A sample of Malathion in the form of 50 per cent emulsifiable concentrate was tested against houseflies *M. nebulo* and mosquitoes *C. fatigans* for its residual toxicity at dosages of 25, 50 and 100 mg. (Malathion) per sq. ft. on glass panels

The test insects were exposed to the treated surfaces for 15 minutes and then kept in separate cages under observation for twenty-four hours after which the percentage mortality was recorded

Malathion was found to be effective for 13 to 14 weeks against mosquitoes and 11 to 13 weeks against flies at the above dosages. Increase in dosage did not proportionately increase the duration of its effectiveness. DDT at 50 mg./sq. ft., when similarly tested, was effective for only five to seven weeks. The formulation, however, has a pungent odour which is rather unpleasant when used in a room.

(*b*) *Diazinon.*—Diazinon is an organic ester of thiophospheric acid (2-isopropyl-4-methyl-pyrimidyl-(6 diethyl ester). It has been reported to be effective against houseflies and mosquitoes, which have developed resistance to chlorinated hydrocarbon insecticides.

A sample of Diazinon, 40 per cent wettable powder, was tested for its residual toxicity at a dosage of 36 mg. Diazinon/sq. ft. (as advised by the firm) applied on glass panels in the same manner as described under Malathion. Diazinon was found to be effective for six weeks against mosquitoes and seven weeks against house-flies. DDT at 50 mg./sq. ft. tested similarly, was also effective for five and seven weeks, respectively. Diazinon has been reported to be comparatively more effective than chlorinated hydrocarbons as a larvicide. Further tests are in progress.

B. Field investigations.

Comparative effectiveness of DDT, BHC, Dieldrin and Malathion residual sprays against mosquitoes.

The comparative effectiveness of the first three insecticides was studied in a number of villages in Madhya Bharat and Vindhya Pradesh in 1954 and preliminary results were reported last year at the Indian Council of Medical Research meetings. The final analysis of the data is briefly summarized below:

The results showed that out of the three types of DDT application (namely, uniform spray of houses with DDT 100 mg./sq. ft., spray of DDT (100 mg./sq. ft.) in the form of bands or strips (1½ ft. wide bands of untreated surfaces were left untreated between successive swaths) and spray of DDT 100 mg./sq. ft. to selective resting place of the vector species (the wall with the entrance door and opposite were left unsprayed). Best results were obtained with uniform application. DDT applied to the selective resting places was found to be more efficacious than that applied in the form of bands. Results on the effect of lime on DDT applications and the comparative efficacy of DDT and dieldrin were inconclusive.

Largescale trials with these four insecticides are being carried out this year in three different States, viz., Madhya Bharat, Vindhya Pradesh

and Madhya Pradesh in collaboration with the malaria organizations in the respective States.

In each State, six areas have been selected and in each of the five areas one particular insecticide has been sprayed. The sixth area has been left untreated for purposes of comparison. Each area consists of a number of villages having a population varying from 3,000 to 10,000.

The following data are being collected from each of the six areas:

A. *Epidemiological:*

1. Pre- and post-spraying spleen and parasite rates in children (2-10 years old).

2. Infant parasite rate every month.

B. Entomological:

1. Comparative density of mosquitoes, anopheline and culicines and the survival rate of those caught in the sprayed areas every week.

2. Behaviour of vector species (*a*) daytime resting places, (*b*) time of entry into human dwellings for feeding and resting and the normal duration of each stay, (*c*) time and frequency of feeding.

Final assessment of the efficacy of each insecticide will be made after the malaria season sometime in December, 1955.

C. SAMPLES OF INSECTICIDES AND SPRAYING EQUIPMENT TESTED.

Insecticides		*Received from*
1. Strobane (Technical)	...	World Health Organization.
2. Pyrethrum extract	...	Messrs. Aikay & Co., Dehradun.
3. 50 per cent BHC water dispersible powder	...	Malaria Organization Coalfield supplied to them (Messrs. Bharat Pulverising Mills Ltd.).
4. 10 per cent shark liver oil	...	Government Shark Liver Oil Factory, Trivandrum.
5. Resitox	...	Dr. L. Kant, A.D.P.H., Malaria Control, Patna.
6. Hexamar BHC 50 per cent		Antimalaria Officer, Ganga Khadar, P.O. Hastinapur, Meerut.
7. DDT 50 per cent	...	" " " "
8. Salvi mosquito repellent	...	Messrs. Salvi Medical Hall, Karnal.
9. BHC 50 per cent water dispersible powder	...	Messrs. Bharat Pulverising Mills Ltd.
10. Dialdrex	...	Messrs. Burmah Shell Oil Storage & Distributing Co. of India Ltd.
11. DDT (Technical)	...	Hindustan Insecticides Ltd.
12. Shalimar Insecticide Lacquer		Shalimar Paint Colour & Varnish Co., Delhi.
13. Thanite	...	Hardcastle Waud & Co. Ltd., Bombay.